DESTRUCTION OF PATHOGENS

APPENDIX 5.1.1.

HYGIENIC PRECAUTIONS

Article 5.1.1.1.

The use of needles and syringes in routine [veterinary] aquatic animal health work in *aquaculture establishments* for procedures such as blood sampling and vaccination should be carried out in a highly professional manner, ensuring that appropriate hygienic precautions are observed.

The intraperitoneal use of unsterilised needles or syringes in *aquatic animals* should be professionally unacceptable.

The use of unsterilised or contaminated equipment (needles, syringes, etc.) or products is especially unacceptable between different *aquaculture establishments* and for live *aquatic animals* that are to be exported. It is a requirement, particularly applicable to *aquatic animals* that are to be exported live, that necessary care be taken to ensure the sterility of all the equipment and products [used] associated with the conditions of certification.

These precautions have particular importance for teams of veterinarians and other aquatic animal health specialists, including vaccination service providers.

The range of organisms capable of being transmitted includes viruses, bacteria and protozoa. The list of infectious agents transmissible in the context of this Appendix continues to expand for all species of *aquatic animals*.

SECTION 5.2.

APPENDIX 5.2.1.

DISINFECTION OF FISH EGGS [WITH IODINE]

Article 5.2.1.1.

Introduction

Although generally effective for decontamination of egg surfaces, the use of [iodophor] *disinfectants*, such as iodophors, cannot be relied upon to prevent vertical transmission of some bacterial (e.g. *Renibacterium salmoninarum*) and viral pathogens (e.g. infectious pancreatic necrosis virus) that may be present within the *egg*.

Article 5.2.1.2.

Conditions of use

The pH of the solutions of the iodophor products must be between 6 and 8. At a pH of 6 or less, the toxicity for *eggs* increases, and at 8 or more, the antiseptic efficacy decreases. It is therefore essential to control the pH, and 100 mg/litre of $NaHCO_3$ must be added to water with a low alkalinity value. It is recommended that the *eggs* be rinsed in fresh water before and after *disinfection*, or that the iodine be neutralised with sodium thiosulfate, and that water free from organic matter be used to prepare the iodophor solution. Generous amounts of this solution should be used and the solution should be replaced when it turns pale yellow and before the colour disappears. One litre of solution at a concentration of 100 mg/litre *disinfectant* will disinfect 2000 salmonid *eggs*. The contact time at this concentration should be no more than 30 minutes.

Finally, in the case of *eggs* that have been transported, the packaging should also be disinfected or, better still, destroyed in a manner that will not pose a contamination or health risk to water and/or other *fish* at the end destination.

Certain precautions must be taken prior to the use of iodophors as products on the market contain a variable quantity of detergents that can give rise to toxic effects. It is therefore recommended that preliminary tests be carried out among the products on the market. It is advisable to build up stocks of the most satisfactory product, but expiry dates must be considered.

Disinfection of *eggs* with iodine can be carried out for the various *fish* species but it is most commonly used for *fish* of the Salmonidae family. For the other species, preliminary tests should be conducted to determine at what egg stage and iodophore concentration [when and at what] disinfection can be carried out safely.

Disinfection of *eggs* of marine species, such as plaice, cod, Atlantic halibut, for which adverse effects have been documented, may be obtained with 400–600 mg/litre glutaraldehyde with a contact time of 5–10 minutes. However, this is not effective against nodaviruses, for which the use of ozone at 1 mg O_3/litre for 30 seconds is recommended. A concentration of ozone of 0.1–0.2 mg O_3/litre for 3 minutes inactivates most pathogenic fish bacteria as well.

Article 5.2.1.3.

Efficacy limits

Disinfection of *eggs* with iodine is ineffective when trying to avoid vertical transmission of infectious pancreatic necrosis, renibacteriosis and even infectious haematopoietic necrosis, for which this

method was recommended initially. The ineffectiveness of iodine has been proved by epidemiological surveys and laboratory tests.

Article 5.2.1.4.

Neutralisation of halogens

See Appendix 5.2.2.

APPENDIX 5.2.2.

DISINFECTION OF FISH FARMS

Article 5.2.2.1.

General principles

The choice of *disinfection* procedures depends on the size, type and nature of the materials and sites to be disinfected. With the exception of the skin of personnel and *eggs*, which must be disinfected with non-corrosive products, the surfaces to be disinfected consist of fabric or woven material (clothes, nets), hard surfaces (plastic, cement) or permeable materials (earth, gravel). *Disinfection* is more difficult for permeable surfaces and requires more time. Table 1 indicates the most common ingredients and the methods to be used on the basis of these criteria.

The use of chemical [methods] products entails the implementation of measures to protect personnel. It is first necessary to protect the skin and eyes from contact with dangerous substances by using impermeable dothing, boots, glasses and a hat. The respiratory tract must be protected by a mask and the operator must not touch any food without having thoroughly washed his/her hands. Finally, the products must be stored in such a way as not to present direct or indirect danger to animal/*fish* or human life.

The material must be thoroughly cleaned before being disinfected.

Ideally, an approval scheme for *disinfection* of products for use in aquaculture should be established. An approval scheme should consider disinfection effect against target pathogens, toxicological and ecotoxicological properties of the products.

Article 5.2.2.2.

Disinfection

See Table 1.

Table 1. *Disinfection and method of use*

Processes	Indications	Method of use *	Comments
Physical			
Desiccation, light	Fish pathogens on earthen bottoms	Dry for 3 months at an average temperature of 18°C	Drying period can be reduced by the use of a chemical disinfectant
Dry heat	Fish pathogens on concrete, stone, iron, ceramic surfaces	Flame-blower, blow-lamp	
Damp heat	Fish pathogens in transportation vehicle tanks	Steam at 100°C or more for 5 minutes	

Table 1 (continued). *Disinfection and method of use*

Processes	Indications	Method of use *	Comments
Physical			
Ultra-violet rays	Viruses and bacteria	10 mJ/cm^2	Minimum lethal dose
Ultra-violet rays	[*Myxosporidian* spores in water] *Myxobolus cerebralis*	35 mJ/cm^2	In order to inactivate all sporoplasm cells in the triactinomyxon stage a dose of 1300 mJ/cm^2 must be used
Ultra-violet rays	Infectious pancreatic necrosis (IPN) and nodavirus (VNN/VER[1]) in water	125 200 mJ/cm^2	
Chemical			
Quartenary ammonia	Virus, bacteria, hands	1 mg/litre for 1 minute	IPN virus resistant
Quartenary ammonia	Gill bacteria, plastic surfaces	2 mg/litre for 15 minutes	
Calcium[a] oxide	Fish pathogens on dried earth-base	0.5 kg/m^2 for 4 weeks	Replace in water and empty disinfected pools keeping the effluents at pH <8.5
Calcium[a] (hypochlorite)	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/litre left to inactivate for several days	Can be neutralised with sodium thiosulfate. See special recommendations
Calcium[a] cyanamide	Spores on earthen bottoms	3000 kg/ha on dry surfaces; leave in contact for 1 month	
Formalin	Fish pathogens in sealed premises	Released from formogenic substances, generally trioxymethylene. Comply with instructions	Nodavirus resistant
Iodine (iodophors)	Bacteria, viruses		See special recommendations
Iodine (iodophors)	Hands, smooth surfaces	>200 mg iodine/litre a few seconds	
Iodine (iodophors)	Eyed eggs	[100 mg iodine/litre for 10 minutes] 100 mg iodine/litre for not more than 30 minutes	
Iodine (iodophors)	Gametes during fertilisation	25 mg iodine/litre for several hours	

1 Viral nervous necrosis/Viral encephalopathy and retinopathy

Iodine (iodophors)	Nets, boots and clothing	200 mg iodine/litre	

Table 1 (continued). *Disinfection and method of use*

Processes	**Indications**	**Method of use ***	**Comments**
Chemical			
Ozone	Sterilisation of water, fish pathogens,	0.2 1 mg/litre for 3 minutes	Costly
Ozone in seawater	Egg disinfection	0.2–1 mg/litre TRO[2] for 0.5–3 minutes	
Ozone in seawater	Surfaces, equipment	0.5–1 mg/litre TRO for 30–60 minutes	
Sodium[a] (hydroxide)	Fish pathogens on resistant surfaces with cracks	Mixture: Sodium hydroxide, 100 g Teepol®, 10 g Calcium hydroxide, 500 g Water, 10 litres Spray, 1 litre/10 m^2 Leave for 48 hours	The most active disinfectant $Ca(OH)_2$ stains the surfaces treated; Teepol® is a tensio-active agent. Turn water on, checking pH
Sodium[a] (hypochlorite)	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/litre. Leave to inactivate for a few days or neutralise with Na thiosulfate after 3 hours	
Sodium[a] (hypochlorite)	Nets, boots and clothing	200 mg available chlorine/litre for several minutes	
Sodium[a] (hypochlorite)	Hands	Rinse with clean water or neutralise with thiosulfate	

a Dangerous See precautions indicated in general recommendations

* The concentrations indicated are those for the active substance. NB: The chemicals must be approved for the prescribed use and used according to the manufacturer's specifications.

Article 5.2.2.3.

Neutralisation of halogens

Chlorine and iodine are highly toxic for *aquatic animals* and, in order to prevent serious accidents that could result from a manipulation error, it is recommended to neutralise these products with sodium thiosulfate five moles of thiosulfate neutralise four moles of chlorine. The molecular proportions are the same for iodine.

2 Total residual oxidant

Accordingly, in order to inactivate chlorine, the amount of thiosulfate should be 2.85 times the amount of chlorine (in grams):

Number of grams of thiosulfate = 2.85 number of grams of chlorine.

For iodine, the amount of thiosulfate should be 0.78 times the amount of iodine in grams:

Number of grams of thiosulfate = 0.78 number of grams of iodine.

It is also possible to prepare a thiosulfate solution at 1% by weight, in which case the neutralising volumes will be as follows (in ml):

1. for chlorine:

   28.5 [number of litres of the disinfecting solution concentration mg/litre] / 100

2. for iodine:

   it is necessary to multiply by 7.8 instead of by 28.5.